ENVIROLOGIX
QUICKTOX KIT FOR QUICKSCAN
AFLATOXIN FREE QUANTITATIVE TEST KIT

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GENERAL INFORMATION

The QuickTox Kit for QuickScan Aflatoxin FREE test method uses lateral flow strip assay technology, and a non-hazardous (water-based) extraction powder procedure that eliminates the use of organic solvents (methanol, ethanol, acetonitrile, etc.) to report aflatoxin results in parts per billion (ppb) in corn and wheat. Other matrices require use of 50% Ethanol (Part No. ACC E26902-1X), 80% Ethanol (Part No. ACC—EE23805-4L), or 84% Acetonitrile (Part no. ACC-EE-19702-4L) for extraction.

The instructions presented in this document cover only the procedure for performing the analytical test for official inspections. For questions regarding this procedure, contact Dr. Ajit Ghosh of the Technology and Science Division by phone at 816-891-0417 or email at Ajit.K.Ghosh@usda.gov.

Refer to the current policies and/or instructions issued by the Policies, Procedures, and Market Analysis Branch (PPMAB) of the Field Management Division for information on use of this test kit in official inspections including sampling, general sample preparation (e.g., grinding and dividing), reporting and certification of test results, laboratory safety, and hazardous waste management. For questions regarding these policies and/or instructions, contact Patrick McCluskey of PPMAB by phone at 816-659-8403 or email at Patrick.J.McCluskey@udsa.gov.

Approved Test Kit Information

Test Kit Vendor:	EnviroLogix Inc. 1-207-797-0300
Test Kit Name:	EnviroLogix QuickTox Kit for QuickScan Aflatoxin FREE
Product Number:	AQ 209 BG
Effective Date of Instructions:	01/07/2015
Instructions Revision Number:	1
Conformance Range:	5 – 100 ppb
Number of Analyses to Cover Conformance Range:	2
Type of Service:	Quantitative
Supplemental Analysis:	No
Approved Commodities:	Corn, corn gluten meal, corn gluten feed, corn germ, corn germ meal, rough rice, oats, barley, corn flour, sorghum, soybean meal, hominy feed, distillers dried grains with solubles (DDGS), delinted cottonseed, rice bran, and wheat.
Extraction method:	For corn and wheat: Shake 50-gram sample with two packets of EB17 extraction powder and 150 milliliters (mL) of distilled or deionized water for 1 minute using an orbital shaker at high speed (~280 rpm). For barley, corn flour, oats, rough rice, sorghum, and soybean meal: Shake 50-gram sample with 100 ml of 50% Ethanol (Part No. ACC E26902-1X) for 1 minute using an orbital shaker at high speed (~280 rpm).

	For rice bran, DDGS, delinted cottonseed, corn gluten meal, hominy feed, and corn germ: Shake 50-gram sample with 200 mL of 50% Ethanol (Part No. ACC E26902-1X) for 1 minute using an orbital shaker at high speed (~280 rpm). For corn germ meal: Shake 50-gram sample with 100 mL of 80% Ethanol for 1 minute using an orbital shaker at high speed (~280 rpm). For corn gluten feed: Shake 50-gram sample with 80 mL of 84% Acetonitrile for 2 minutes using an orbital shaker at high speed (~280 rpm).
Test Format:	Lateral Flow Strip
Detection Method:	QuickScan System (reader)

EXTRACTION PROCEDURES

a. Extraction Procedure for Corn

(1) Transfer 50.0 ± 0.2 grams ground sample into an extraction container.

(2) Add the contents of 2 extraction packages of EB17 to the 50-g corn sample and shake by hand to evenly distribute the extraction buffer components throughout the sample.

(3) Add 150 mL of distilled or deionized water and securely seal the extraction container; ensure that the sample is fully wetted.

(4) Cover the extraction container and shake at high speed (~280 rpm) on an orbital shaker for 1 minute.

Shake mechanically

(5) Filter the sample through an approved coffee filter (EnviroLogix Part No. 11434) into a clean collection vessel. This is the **filtered (clarified) sample extra**ct for testing.

Filter extract with an approved coffee filter

(6) Proceed to **Test Procedures.**

b. Extraction Procedure for Wheat

(1) Transfer 50.0 ± 0.2 grams ground sample into an extraction container

(2) Add 150 mL of distilled/deionized water; seal the extraction container and wet the wheat sample thoroughly before adding the contents of 2 extraction packages of EB17.

(3) Cover the extraction container and shake at high speed (~280 rpm) on an orbital shaker for 1 minute.

(4) Fill a micro-centrifuge tube with extract. Centrifuge for 30 seconds at 2000 x g (not rpm). The top layer containing the aflatoxin residues will be used for testing. This is the **centrifuged sample extract** and ready for testing.

(5) Proceed to the **Test Procedure**s.

c. Extraction Procedure for Rice Bran, DDGS, Delinted Cottonseed, Corn Gluten Meal, Corn Germ, and Hominy Feed

(1) Transfer 50.0 ± 0.2 grams ground sample into an extraction container.

(2) Add 200 mL of 50% Ethanol; seal the extraction container and wet the sample thoroughly.

(3) Shake on an orbital shaker at high speed (~280 rpm) for 1 minute.

(4) Fill a micro-centrifuge tube with extract. Centrifuge for 1 minute at 2000 x g (not rpm). The top layer containing the aflatoxin residues will be used for testing. This is the **centrifuged sample extract** for testing.

(5) Proceed to **the Test Procedure**s.

d. Extraction Procedure for Barley, Corn Flour, Oats, Rough Rice, Sorghum, and Soybean Meal

(1) Transfer 50.0 ± 0.2 grams ground sample into an extraction container.

(2) Add 100 mL of 50% Ethanol; seal the extraction container and wet the sample thoroughly.

(3) Shake on an orbital shaker at high speed (~280 rpm) for 1 minute.

(4) Fill a micro-centrifuge tube with extract. Centrifuge for 1 minute at 2000 x g (not rpm). The top layer containing the aflatoxin residues will be used for testing. This is the **centrifuged sample extract** for testing.

(5) Proceed to the **Test Procedure**s.

e. <u>Extraction Procedure for Corn Germ Meal</u>

(1) Transfer 50.0 ± 0.2 grams ground sample into an extraction container.

(2) Add 100 mL of 80% Ethanol; seal the extraction container and wet the sample thoroughly.

(3) Shake on an orbital shaker at high speed (~280 rpm) for 1 minute.

(4) Settle the extraction mixture for 2 minutes. This is the **clarified sample extract** for testing.

(5) Proceed to the **Test Procedure**s.

f. <u>Extraction Procedure for Corn Gluten Feed</u>

(1) Transfer 50.0 ± 0.2 grams ground sample into an extraction container.

(2) Add 80 mL of 84% Acetonitrile; seal the extraction container and wet the sample thoroughly.

(3) Shake on an orbital shaker at high speed (~280 rpm) for 2 minutes.

(4) Settle the extraction mixture for 1 minute. This is the **clarified sample extract** for testing.

(5) Proceed to the **Test Procedure**s.

TEST PROCEDURES

Note that the lot specific Multi -Matrix Barcode Card must be scanned into the QuickScan System before analyses of rice bran, DDGS, corn gluten meal, delinted cottonseed, corn germ, corn germ meal, corn gluten feed, barley, corn flour, oats, rough rice, sorghum, soybean meal and hominy feed. Ensure you have scanned in the Multi-Matrix Barcode Card that corresponds to the device lot being run. QuickScan software version 4.5 or higher is needed. The barcode card is not required for analyses of corn and wheat. If needed, follow operation manual and/or Envirologix guide for setting up QuickScan reader system.

a. <u>Sample Preparation and Analysis for 5 to 30 ppb quantitation range for corn, wheat, rice bran, DDGS, corn gluten meal, delinted cottonseed, corn germ, and hominy feed.</u>

(1) Using a calibrated pipette with new pipet tip, place 100 microliters (µL) of assay buffer DB5 into a reaction vial. Take care not to contaminate buffer; use a **new tip** for each vial containing buffer.

(2) Using a new pipette tip, add 100 µL of clarified sample extract to the reaction vial containing 100 µL of buffer.

(3) Mix buffer and sample extract thoroughly by pipetting up and down 5 times with the pipette tip.

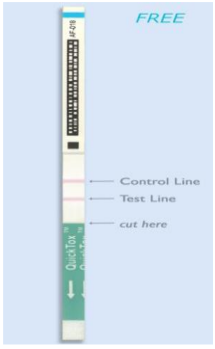
(4) Place an assay test strip into the vial with the arrow end down.

(5) Allow the test strip to develop for **4 minutes** for corn.

(6) Allow the test strip to develop for 5 **minutes for** wheat, DDGS, corn gluten meal, rice bran, corn germ and hominy feed.

(7) Allow the test strip to develop for **7 minutes** for delinted cottonseed.

(8) After designated incubation time immediately cut off the arrow end of the strip.



Cut strip and place in QuickScan reader immediately —no drying step!

(9) Place the test strip face down in the carrier with the barcoded end closest to the handle.

(10) Push the Read Test button. If you scanned the multi-matrix barcode card (required for rice bran, DDGS, delinted cottonseed, corn gluten meal, corn germ, and hominy feed) you will be prompted to select the matrix group that corresponds to the sample run.

(11) Results are displayed; ensure the pull down tab indicates 1:1.

(12) Make the appropriate sample notations on the results screen window and work record.

(13) Results are archived in the Datalog file.

Note: See Interpreting the Lateral Flow Test Strip with QuickScan Reader section for information.

b. <u>Sample Preparation and Analysis for 5 to 30 ppb quantitation range for barley, corn flour, oats, rough rice, sorghum, soybean meal, and corn germ meal.</u>

(1) Using a calibrated pipette with new pipet tip, place 200 microliters (µL) of assay buffer DB5 into a reaction vial. Take care not to contaminate buffer. Use a **new tip** for each vial containing buffer.

(2) Using a new pipette tip, add 100 µL of clarified sample extract to the reaction vial containing 200 µL of buffer.

(3) Mix buffer and sample extract thoroughly by pipetting up and down 5 times with the pipette tip.

(4) Place an assay test strip into the vial with the arrow end down.

(5) Allow the test strip to develop for 5 **minutes**

(6) Immediately cut off the arrow end of the strip.

(7) Place the test strip face down in the carrier with the barcoded end closest to the handle.

(8) Push the Read Test button. You will be prompted to select the matrix group that corresponds to the sample run.

(9) Results are displayed; ensure the pull down tab indicates 1:1.

(10) Make the appropriate sample notations on the results screen window and work record.

(11) Results are archived in the Datalog file.

c. <u>Sample Preparation and Analysis for 5 to 30 ppb quantitation range corn gluten feed</u>

(1) Using a calibrated pipette with new pipet tip, place 500 microliters (µL) of assay buffer DB5 into a reaction vial. Take care not to contaminate buffer.

(2) Using a new pipette tip, add 100 µL of clarified sample extract to the reaction vial containing 500 µL of buffer.

(3) Mix buffer and sample extract thoroughly by pipetting up and down 5 times with the pipette tip.

(4) Transfer 200 µL of the buffer-sample mixture to a new vial.

(5) Place an assay test strip into the vial with the arrow end down.

(6) Allow the test strip to develop for 5 **minutes.**

(7) Immediately cut off the arrow end of the strip.

(8) Place the test strip face down in the carrier with the barcoded end closest to the handle.

(9) Push the Read Test button. You will be prompted to select the matrix group that corresponds to the sample run (AF MG5).

(10) Results are displayed; ensure the pull down tab indicates 1:1.

(11) Make the appropriate sample notations on the results screen window and work record.

(12) Results are archived in the Datalog file.

Note: See Interpreting the Lateral Flow Test Strip with QuickScan Reader section for information.

d. <u>Sample Preparation and Additional Dilution Procedure for 30 to 100 ppb quantitation range for corn and wheat.</u>

(1) Prepare Dilution Solution by dissolving one EB17 packet in 300 mL of distilled/deionized water. Label, date and document technician prepared by. (This is not a clear solution and can be stored at ambient temperature for 30 days). Thoroughly mix before use.

(2) Using a calibrated pipette with a new pipette tip, add 500 µL of Dilution Solution to a reaction vial or other suitable container.

(3) Using a new pipette tip, add 100 µL of filtered sample extract (for corn) or centrifuged sample extract (for wheat) to the 500 µL Dilution Solution and mix by pipetting up and down 5 times. This is the **Diluted Sample Extract** for testing.

(4) Add 100 µL of assay buffer DB5 to a new reaction vial.

(5) Add 100 µL of the diluted sample extract to the reaction vial and mix by pipetting up and down 5 times.

(6) Place an assay strip into the reaction vial with the arrow end down.

(7) Allow the strip to develop for **4 minutes for corn and 5 minutes for wheat**. Remove after development time and cut-off the arrow end of the strip.

(8) Place the test strip face down in the carrier with the barcoded end closest to the handle and insert the carrier into the reader.

(9) Push the read test button. If you scanned the multi-matrix barcode card you will be prompted to select the matrix group that corresponds to the sample run (On Strip Matrix Group).

(10) Results are displayed; ensure the ‘Select Dilution’ pull down tab indicates 1:6 on the

Results Screen.

(11) Make the appropriate the appropriate sample notation(s) on the results screen window and work record.

(12) Results are archived in the Datalog file.

Note: <u>Do not report test results below 30 ppb or above 100 ppb using the Additional Dilution Procedure.</u>

e. <u>Sample Preparation and Additional Dilution Procedure for 30 to 100 ppb quantitation range for rice bran, DDGS, corn gluten meal, corn germ, delinted cottonseed, and hominy feed.</u>

(1) Using a calibrated pipette with a new pipette tip, add 500 µL of 50% Ethanol to a clean vial.

(2) Using a new pipette tip, add 100 µL of centrifuged sample extract to 500 µL of 50% Ethanol and mix by pipetting up and down 5 times. This is the **Diluted Sample Extract** for testing.

(3) Add 100 µL of assay buffer DB5 to a new reaction vial.

(4) Add 100 µL of the diluted sample extract to the reaction vial and mix by pipetting up and down 5 times.

(5) Place an assay strip into the reaction vial with the arrow end down.

(6) Allow the strip to develop for **5 minutes for** DDGS, corn germ, corn gluten meal, rice bran, and hominy feed.

(7) Allow the test strip to develop for **7 minutes** for delinted cottonseed.

(8) Remove after the development time and cut off the arrow end of the strip.

(9) Place the test strip face down in the carrier with the barcoded end closest to the handle.

(10) Push the Read test button.

(11) You will be prompted to select the matrix group that corresponds to the sample run.

(12) Results are displayed; ensure the 'Select Dilution' pull down tab indicates 1:6 on the Results Screen.

(13) Make the appropriate sample notation(s) on the results screen window and work record.

(14) Results are archived in the Datalog file.

Note: <u>Do not report test results below 30 ppb or above 100 ppb using the Additional Dilution Procedure.</u>

f. <u>Sample Preparation and Additional Dilution Procedure for 30 to 100 ppb quantitation for barley, corn flour, oats, rough rice, sorghum, soybean meal, and corn germ meal</u>

(1) Using a calibrated pipette with a new pipette tip, add 500 µL of 50% Ethanol to a clean vial. (Only for corn germ meal use 80% Ethanol).

(2) Using a new pipette tip, add 100 µL of centrifuged (clarified) sample extract to 500 µL of 50% Ethanol (only for corn germ meal use 80% Ethanol) and mix by pipetting up and down 5 times. This is the **Diluted Sample Extract** and ready for testing.

(3) Add 200 µL of assay buffer DB5 to a new reaction vial.

(4) Add 100 µL of the diluted sample extract to the reaction vial and mix by pipetting up and down 5 times.

(5) Place an assay strip into the reaction vial with the arrow end down.

(6) Allow the strip to develop for **5 minutes**

(7) Remove after the development time and cut off the arrow end of the strip.

(8) Place the test strip face down in the carrier with the barcoded end closest to the handle.

(9) Push the Read test button.

(10) You will be prompted to select the matrix group that corresponds to the sample run.

(11) Results are displayed; ensure the 'Select Dilution' pull down tab indicates 1:6 on the Results Screen.

(12) Make the appropriate sample notation(s) on the results screen window and work record.

(13) Results are archived in the Datalog file.

Note: <u>Do not report test results below 30 ppb or above 100 ppb using the Additional Dilution Procedure.</u>

g. <u>Sample Preparation and Analysis for 30 to 100 ppb quantitation range corn gluten feed</u>

(1) Place 500 µL 84% acetonitrile into a vial.

(2) Add 100 µL of clarified extract and mix by pipetting up and down 5 times. This is the diluted sample extract.

(3) Using a calibrated pipette with new pipet tip, place 500 microliters (µL) of assay buffer DB5 into a new reaction vial.

(4) Using a new pipette tip, add 100 µL of diluted sample extract to the reaction vial containing 500 µL of assay buffer DB5.

(5) Mix buffer and sample extract thoroughly by pipetting up and down 5 times with the pipette tip.

(6) Transfer 200 µL of the buffer-sample mixture to a new vial.

(7) Place an assay test strip into the vial with the arrow end down.

(8) Allow the test strip to develop for 5 **minutes**

(9) Immediately cut off the arrow end of the strip.

(10) Place the test strip face down in the carrier with the barcoded end closest to the handle.

(11) Push the Read Test button. You will be prompted to select the matrix group that corresponds to the sample run.

(12) Results are displayed; ensure the 'Select Dilution' pull down tab indicates 1:6 on the Result Screen.

(13) Make the appropriate sample notations on the results screen window and work record.

(14) Results are archived in the Datalog file.

Note: See Interpreting the Lateral Flow Test Strip with QuickScan Reader section for information.

h. Interpreting the Lateral Flow Test Strip with QuickScan System.

Development of a Control Line within stated development times indicates that the strip has functioned properly. Any strip that does not develop a Control Line should be discarded. A second preparation of the extract (using a fresh buffer dilution) should be made and tested using another test strip.

(1) Place the test strip face down in the carrier with the barcoded end closest to the handle.

(2) Insert the carrier into the reader and the strips are read by clicking on the "Read Test" area of the screen.

(3) A pull down menu will appear if the Multi-Matrix barcode card was scanned into the reader. Use the pull down menu to select the appropriate matrix group ID for the sample run. Follow the operational manual and/or Envirologix guide for setting up QuickScan reader system.

Matrix Group ID	**Matrices**
On Strip Matrix Group	corn, wheat
AF MG2	corn germ, delinted cottonseed, DDGS, rice bran
AF MG3	corn gluten meal, hominy feed
AF MG5	corn gluten feed
AF MG6	corn germ meal
AF MG7	rough rice, oats, sorghum
AF MG8	corn flour, soybean meal, barley

SUPPLEMENTAL ANALYSIS

Supplemental analysis is a procedure followed when a result is observed above the upper limit of the conformance range used in GIPSA's test kit performance evaluation. The conformance range for performance evaluation of quantitative aflatoxin test kits is 5 – 100 ppb. Therefore, supplemental analysis would be performed for a result above 100 ppb. **Supplemental analysis is not performed with this test kit.**

REPORTING AND CERTIFYING TEST RESULTS

Refer to the current instructions issued by the Policies, Procedures, and Market Analysis Branch of the Field Management Division for reporting and certification of test results. For questions regarding these instructions, contact Patrick McCluskey (816-659-8403 or Patrick.J.McCluskey@udsa.gov).

STORAGE CONDITIONS AND PRECAUTIONS

a. Storage Conditions

Test kits should be refrigerated between 36°- 48°F. Prolonged exposure to high temperatures may adversely affect the test results. Do not open the desiccated canister until ready to use the strips.

b. Precautions

(1) Strips must be read wet promptly at four (4) minutes for **corn**; five (5) minutes for **wheat, barley, corn flour, corn germ, corn germ meal, corn gluten feed, oats, rough rice, sorghum, soy bean meal, DDGS, corn gluten meal and rice bran and hominy feed**; and seven (7) minutes for **delinted cottonseed** to ensure accurate results.

(2) Do not treat the corn and wheat liquid extracts or the extraction labware with a bleach solution. The Extraction Packet powder is incompatible with strong oxidizers.

(3) Follow your facility's safety procedures for disposal of samples and extracts potentially containing or known to contain aflatoxin(s).

(4) This product is currently not officially approved for use in testing any other crops beyond corn, corn gluten meal, corn gluten feed, corn germ, corn germ meal, rough rice, oats, barley, corn flour, sorghum, soybean meal, hominy feed, distillers dried grains with solubles (DDGS), delinted cottonseed, rice bran, and wheat.

(6) Proper and thorough mixing, along with accurate pipetting, are essential to accurate results.

(7) Protect all components from hot or cold extremes of temperature when not in use. Do not leave in direct sunlight or in vehicle.

(8) **IMPORTANT:** EB17 extraction powder should be considered flammable and an irritant (MSDS available at www.envirologix.com/MSDS-AQ209.pdf). Avoid inhaling powder or contact with the skin, eyes, or clothing. Wear personal protective equipment including safety glasses, gloves, mask, and a lab coat when handling.

(9) Keep powder away from heat, sparks and open flame.

(10) **IMPORTANT:** Ethanol and acetonitrile are flammable and toxic. Avoid inhaling vapors or contact with the skin, eyes, or clothing. Wear personal protective equipment including safety glasses, nitrile gloves (not latex), a vapor mask and a lab coat when handling. Keep containers tightly closed and away from heat, sparks and open flame. Observe any applicable regulations when disposing of samples and kit reagents.

(11) Observe any applicable regulations when disposing of samples and kit reagents.

(12) Do not use the test kits beyond the noted expiration date.

EQUIPMENT AND SUPPLIES

a. Materials Provided

(1) QuickScan System (ACC131).

b. Materials Recommended But Not Provided

(1) Mini-Pet pipette (ACC041).

(2) Sample cups with lids (ACC 012-50).

(3) Distilled or deionized water.

(4) 50% Ethanol (ACC E26902-1X).

(5) 80% Ethanol- ACC-EE23805-4L 11963

(6) 84% Acetonitrile ACC-EE19702-4L 11932

(7) Approved coffee filter (ACC 11434).

(8) Laboratory balance.

(9) Sample Grinder.

(10) Graduated cylinder (150 – 200 mL).

(11) Orbital/rotary shaker.

(12) Micro-centrifuge (ACC 064 E).

(13) Dilution set – extra tips and vials for 100 dilutions for test samples above 30 ppb (ACC080).

(14) 50 gram sample extraction set – additional EB17 extraction packets and sample extractions bags (ACC035).

(15) Dilution set – disposables and extraction powder for 100 dilutions (ACC034).

(16) Centrifugation set – disposables for 50 tests (ACC 010).

REVISION HISTORY

Revision 1 (01/07/2015)

- Correct Acronym of Policies, Procedures, and Market Analysis Branch (PPMAB) has been used.
- Phone number of Patrick McCluskey (816-659-8403) has been corrected.

Revision 0 (12/12/2014)